

Appl. No. : 09/579,327  
Filed : May 25, 2000

### AMENDMENTS TO THE CLAIMS

Claims 1-39. (Previously cancelled)

40. (Cancelled)

41. (Previously amended) The method of Claim 67 wherein said removal of insoluble components is by centrifugation.

42. (Previously added) The method of Claim 41 wherein said centrifugation is at 10,000xg for about 20 minutes.

43. (Currently amended) A The method of Claim 40 further for producing an immune stimulating composition comprising:

treating bacteria containing peptidoglycan with an acid treatment solution;

heating at about 100°C during said acid treatment;

removing insoluble components from the solution resulting from said treating;

saving the remaining solution and adjusting the pH to a physiologically acceptable

pH;

testing said solution for immune-stimulating activity; and

obtaining thereby an immune stimulating composition.

44. (Previously added) The method of Claim 43 wherein said heating is for about 2 hours.

45. (Previously amended) The method of Claim 67 wherein said acid is selected from the group consisting of acetic acid, hydrochloric acid, and sulfuric acid.

46. (Previously amended) The method of Claim 67 wherein said acid is acetic acid.

47. (Previously amended) The method of Claim 67 wherein said bacteria containing peptidoglycan is *Lactobacillus*.

48. (Previously added) The method of Claim 47 wherein said bacteria is *L. fermentum*.

49. (Previously amended) The method of Claim 67 further comprising ultrafiltration of said remaining solution.

50. (Previously amended) The method of Claim 67 further comprising removing the lipids from said remaining solution.

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51. (Previously added) The method of Claim 50 wherein said lipids are removed with chloroform.

52. (Currently amended) ~~A The method for producing an immune stimulating composition of Claim 40 further comprising: trichloroacetic acid precipitation of said remaining solution:~~

treating bacteria containing peptidoglycan with an acid treatment solution;  
removing insoluble components from the solution resulting from said  
treating;  
precipitating said solution with trichloroacetic acid;  
saving the remaining solution from said precipitation with trichloroacetic  
acid;  
adjusting the pH to a physiologically acceptable pH;  
testing said solution for immune-stimulating activity; and  
obtaining thereby an immune stimulating composition.

53. (Currently amended) ~~A The method for producing an immune stimulating composition of Claim 40 further comprising: lyophilization of said remaining solution.~~  
comprising:

treating bacteria containing peptidoglycan with an acid treatment solution;  
removing insoluble components from the solution resulting from said  
treating;  
saving the remaining solution and adjusting the pH to a physiologically  
acceptable pH;  
lyophilizing said solution;  
testing said lyophilized solution for immune-stimulating activity; and  
obtaining thereby an immune stimulating composition.

54. (Cancelled)

55. (Cancelled)

56. (Cancelled)

57. (Currently amended) ~~A The method of claim 40 wherein said for producing an immune stimulating composition has a final pH of about 3.0. comprising:~~

treating bacteria containing peptidoglycan with an acid treatment solution having a final pH of about 3.0;

removing insoluble components from the solution resulting from said treating;

saving the remaining solution and adjusting the pH to a physiologically acceptable pH;

testing said solution for immune-stimulating activity; and

obtaining thereby an immune stimulating composition.

58. (Previously amended) The method of claim 67 wherein said composition has a final pH of about 5.3.

59. (Previously amended) The method of Claim 67, wherein said testing is performed by measuring at least one of the parameters selected from the group consisting of: lymphocyte proliferation, cytokine production, and dendritic cell maturation.

60. (Previously amended) A method for producing an immune stimulating composition comprising:

treating bacteria containing peptidoglycan with an acid treatment solution having a final pH of about 2.0;

removing insoluble components from the solution resulting from said treating;

saving the remaining solution and adjusting the pH to a physiologically acceptable pH; and

obtaining thereby an immune stimulating composition.

61. (Previously amended) A method for producing a peptidoglycan extract from bacteria comprising:

heating a Gram positive bacteria in a solution comprising water and acid at a final pH of about 2.0, wherein said solution is free of added raffinose and added enzymes;

removing insoluble particles from the solution resulting from said heating;  
and

adjusting the pH of the remaining solution to about 7.0 obtaining thereby an immune stimulating composition.

62. (Cancelled)
63. (Previously amended) The method of Claim 60 wherein said bacteria containing peptidoglycan is *Lactobacillus*.
64. (Previously amended) The method of Claim 60 further comprising removing lipids from said remaining solution.
65. (Previously amended) The method of Claim 60 further comprising ultrafiltration from said remaining solution.
66. (Previously amended) The method of Claim 60 further comprising trichloroacetic acid precipitation from said remaining solution.
67. (Previously added) A method for producing an immune stimulating composition comprising:
- treating bacteria containing peptidoglycan with an acid treatment solution having a final pH of about 2.0;
  - removing insoluble components from the solution resulting from said treating;
  - saving the remaining solution and adjusting the pH to a physiologically acceptable pH;
  - testing said solution for immune-stimulating activity; and
  - obtaining thereby an immune stimulating composition.
68. (Previously added) The method of Claim 67, wherein said immune stimulating composition is in a form suitable for injectable administration.
69. (Previously added) The method of Claim 67 further comprising heating at about 100°C during said acid treatment.
70. (Previously added) The method of Claim 69 wherein said heating is for about 2 hours.
71. (Previously added) The method of Claim 67 further comprising removing lipids from said remaining solution.
72. (Previously added) The method of Claim 67 further comprising ultrafiltration from said remaining solution.
73. (Previously added) The method of Claim 67 further comprising trichloroacetic acid precipitation from said remaining solution.